THE
BIOLOGICAL BULLETIN

INVESTIGATION ON THE ECOLOGY AND RESPIRATORY RESPONSES OF THE HEMICHORDATE *PTYCHODERA FLAVA* TO TIDAL CYCLES AND SALINITY CHANGES

JAYAPAUL AZARIAH, M. MOHAMED ISMAIL, AND M. AHMED NAJIB

Department of Zoology, University of Madras, Madras-600005, India

Different aspects of the biology of Hemichordates have been studied by a number of workers whose findings have been reviewed by Hyman (1959) and Barrington (1965). In recent years there have been some studies on the chemical constituents of Hemichordates (De Jorge, Petersen and Sawaya 1967; Ashworth and Cormier, 1967; De Jorge and Petersen, 1968a, b; Krishnan and Govindarajulu, 1968; Macha, 1969; Petersen and Longhi, 1971), but physiological aspects have received less emphasis. This may largely be due to "their sluggish responses and tendency to break into pieces on handling" which render them as "poor objects for laboratory experiments" designed to study their physiology (Hyman, 1959, p. 147). The paucity of physiological studies on Hemichordates prompted this investigation.

The occurrence of *Ptychodera flava* in the intertidal sheltered waters of Krusadi Island has been recorded by many workers (Ramanujam, 1935; Kuriyan, 1949; Sundara Rao and Ranga Rao, 1949). Rao (1954a, b; 1955a, b) studied some aspects of distribution, taxonomy, anatomy and development. Work on the physiology of Indian enteropneusts is hampered by their localized distribution around Krusadi Island and lack of good laboratory facilities there. It was, therefore, thought worthwhile to study some aspects of their respiratory physiology by transporting necessary laboratory equipment to Krusadi Island. Further, an attempt has been made to study their respiratory responses with special reference to salinity changes, since it is known (Jayaraman, 1954; Muthu, 1956; Udayavarma and Gangadhara Reddy, 1959) that there is a seasonal cycle in the salinity of the waters of the Bay of Bengal, with low salinity during the months of November and December. Some aspects of the ecology of *P. flava* are also considered in this paper.

MATERIALS AND METHODS

Specimens of *Ptychodera flava* were collected from the lagoon area of the Galaxea reef during low tide by shoveling the sand gently with spread-out fingers. They were gently removed from the adhering mucous coat, cemented with sand, by repeated washing in sea water. Specimens were brought to the Krusadi Island Laboratory and kept under observation during the process of defecation. After 2.5 hours of collection, entire animals with their gut cleared of particles were used for experimental studies.

The apparatus used for the determination of oxygen consumption was a simple continuous flow system described by Fry and Hart (1948) and modified by Job (1955) and Azariah (1969; and unpublished). Sea water filtered with spun glass (Brosiam) wool and with maximum oxygen saturation was used. The animal (respiration) chambers were housed in an insulated water-bath, kept closed throughout the course of experiment in order to eliminate the influence of external factors. After 1.5 hours from the start of the experiment, half-hourly or hourly observations were made on solitary specimens. The volume of water flushing through the animal chamber per hour was calculated after every reading to arrive at the amount of oxygen uptake by the animal. Rate of flow did not influence the rate of oxygen uptake, but care was taken to keep the nozzle from clogging. Samples for oxygen content estimation were drawn from the outlet in bottles with approximate volumes of 15 ml. The initial oxygen concentration of water gaining entry to the respiration chambers was obtained from water-flushing a control respiration chamber without an animal. Oxygen analyses were made by the Winkler's method using $N/100$ sodium thiosulphate standardized against $N/100$ potassium dichromate. Experiments were conducted at $25 \pm 0.5°$ C using sea water of $30.5\%_c$. Dry weights of the specimens were determined, and the rates of oxygen uptake were then calculated.

Individual observations were made on a total of 18 specimens at two different periods of the year. During January, 1974, experiments were performed on six animals; observations on three of these were related to the effect of salinity on the oxygen uptake. In the month of September, 1974, the animals were found to bear mature gonads, and hence only female specimens were chosen for the experiments as they were found in large numbers and easily distinguishable externally (Rao, 1954b).

In order to analyze the data statistically, the following procedure was adopted. The duration between a high tide peak and the succeeding low tide peak was divided equally. This was repeated for other high and low tide peaks. The sectors on either side of peak high tide and low tide were taken as the periods of high tide and of low tide, respectively. Data falling within such a sector were pooled, and the average was taken. The average values of all high tide and low tide periods during an experiment were added separately, and their mean represented the amount of oxygen uptake by an animal during the high or low tide period in the course of a single experiment. The values so obtained were used to illustrate the relationship between body size and oxygen consumption. Such a treatment may eliminate the influence of the phases of the habitat tidal cycle, if any, on oxygen uptake. The paired high tide and low tide averages of an animal were employed in the analysis of significance of variance using Student's *t* test.

In order to study the effect of salinity, the constant-level bottle was flushed with sea water of 20.5‰ salinity by opening the stopper in the tube connecting the reservoir containing the experimental medium (20 ‰) and closing the one in the reservoir with 30.5‰ salinity. The sea water in the constant level bottle was replaced within two minutes by increasing the inflow of water from the reservoir.

Results

Ecological notes

Galaxea lagoon has water heights of about 2–3 feet during high tide, and less than 6 inches at low tide. *P. flava* leaves fecal castings on the surface of the sandy substratum, appearing as small hillocks of about 1.0 to 1.5 cm in height, with a centrally located burrow opening when submerged under water. The posterior anal end of the animal is seen protruding outside the substratum to a distance of about 0.5 to 3.0 cm more often than the anterior proboscis region. This makes the tail end opening of the burrow more prominent than the other. The proboscis is seen to extend outside the burrow up to the collar region. Freshly dug out animals are always covered by a thin mucous coat cemented with sand grains, which makes it difficult to distinguish them from the substratum. In the burrow, however, such a coat was not seen, and animals moved freely within their burrows. Secretion of a mucous coat may be an adaptation to avoid predation since it is reported that they are eaten by fish (Devanesan and Chacko, 1942) and found to lie on the substratum (Hyman, 1959).

Rao (1954b) reported the occurrence of *Edwardsia* (Anthozoa, Coelenterata) and sand-dwelling copepods, such as *Paramesochra arenicola, P. wilsoni* and *Emertonia minuta* (Crustacea, Arthropoda) in the area with *Ptychodera flava*. Forms like *Holothuria scabra* (Holothuroidea, Echinodermata) and hermit crabs in dead *Cerithium* shells have also been found. The area is dominated by *Cymadocea* sp. (sea-grass) with algae, such as *Acanthophora* sp., *Laurencia* sp., *Geladium* sp. and *Helimeda opuntioides* also being represented.

Ten acorn worms were taken to Madras (675 km distant) and were then reared in glass tanks containing 2–3 inches of sea water without sandy substratum. All were healthy and found to lie passively and extended, usually on their ventral side with the genital folds of either side meeting at the mid-dorsal line. The sea water was changed on alternate days. In the absence of sand the animals mainly depended on particulate matter for food and as a result a small amount of dark amber colored fecal matter was passed.

Nine acorn worms were then transferred to a glass tank containing an inch of sand collected from the inshore waters of Madras. On contact with the sand a slow wriggling movement of the body was observed in all the animals, with maximum activity in the proboscis region. As reported by Hyman (1959), there was copious secretion of mucus. Sand particles stream backwards from the proboscis region and get entangled in the thin film of mucus to form a tube-like structure around the animals. Formation of such a sand coat was continued until the region between the proboscis and a portion of the trunk region was covered. Then the proboscis was withdrawn inside the tube and did not make its appearance thereafter. Later, the withdrawal inside the tube is followed by deeper burrowing.

TABLE I

Relationship between body size, time taken for defecation and gut contents in P. flava.

Number	Breadth of collar (mm)	Time taken for defecation (minutes)	Weight of gut contents (mg)
1	3	30	240.4
2	5	45	293.6
3	5	25	360.0
4	5	95	358.0
5	5	90	389.6
6	10	95	1387.8

Table I shows the relationships between the size of the animal and the time taken for the gut to empty, together with the weight of the gut contents. It may be seen that the smallest specimen takes thirty minutes, and the largest takes about ninety-five minutes to empty its gorged gut.

Another facet of ecological interest is that of the differences between the pH of the sea water and the pH of the gut content. The pH of the sea water was between 7.0 and 7.2. After blotting, a fully fed animal was held vertically by the collar region and a portion of the trunk region was cut allowing free discharge of gut contents directly onto pH papers, which indicated a pH of 9.0. From the work of Barrington (1940) it is known that the amylase contained in body extracts was active between pH 5.5 and 8.0. The significance of the high alkaline pH in the gut compared to the near neutral pH of the habitat medium is not clear. Further work is proposed.

Rate of oxygen consumption as a function of body size and maturity stage

Figure 1 (solid squares) shows the relationship between the rate of oxygen consumption of six *P. flava* during the high tide period, (see materials and methods) and body dry weight; the animals range in body weight from 89.0 mg to 325.6 mg. The rate of oxygen uptake under constant conditions was found to vary between individuals and on the basis of weight, the smallest animal consumes 0.7629 ml/g dry wt/hr, which is about one and one-half times more than that of the largest. Individual variation in the amount of oxygen consumption of an animal, for example, 89.0 mg shows a wide range of variation from 0.0191 to 0.1056 ml/hr (Table III) which may be due to the correlation between the respiratory responses and phases of the habitat tidal cycle.

A linear regression equation calculated from the observed data (Y on x) gave the following regression coefficient: $Y = -521.99x + 469.46$, where $Y =$ the weight of the animal and $x =$ the rate of oxygen uptake.

Data obtained on the rate of oxygen consumption of twelve specimens of *P. flava* during the late maturity stage—mature and spawning—are presented in Figure 1. Data on low tide periods and high tide periods are plotted separately. In both the periods, the rate of oxygen consumption of the smallest animal is higher than that of the largest, and the increase is about six times during the low tide period and eight times during the high tide period. The range of individual variation in oxygen uptake during the course of each experiment is wide. The
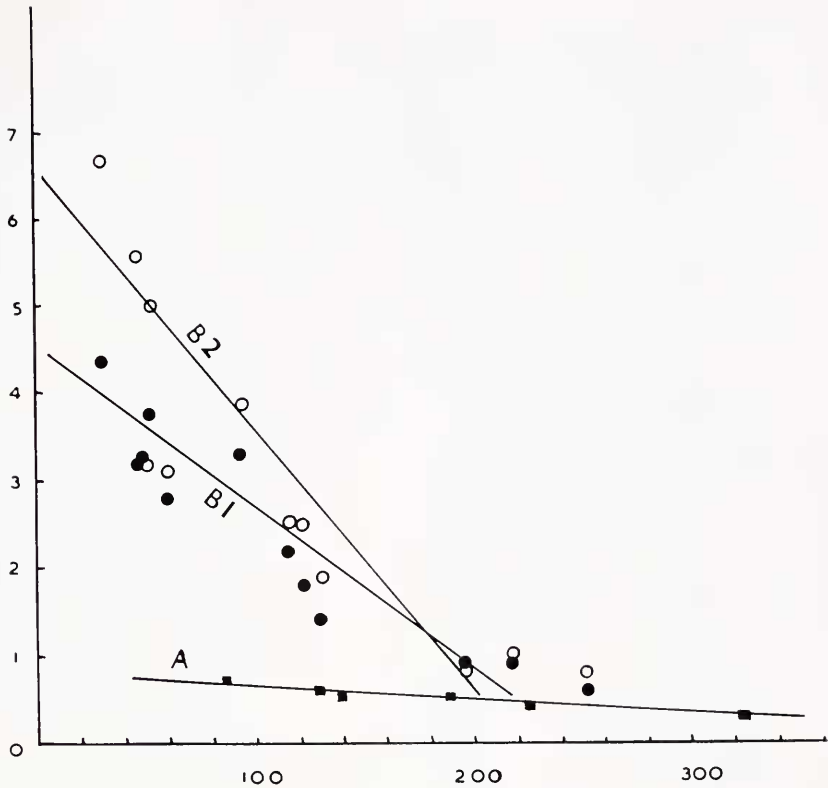
FIGURE 1. Rate of oxygen uptake of *P. flava* in relation to body weight; abscissa represents dry body weight in mg; ordinate, rate of oxygen uptake in ml/g/hr. Solid squares indicate the rate of oxygen uptake of *P. flava* during high tide periods in non-breeding season. Solid circles indicate the rate of oxygen uptake of *P. flava* during low tide periods in breeding season. Open circles indicate the rate of oxygen uptake of *P. flava* during high tide periods in breeding season. Solid lines A, B1, and B2 are the computed regression lines.

straight lines, B1 and B2, were derived statistically from low and high tide periods respectively; the regression equations being $Y = -53.63x + 244.78$ (B1) and $Y = -33.25x + 218.10$ (B2), respectively. The metabolic rate of the animals during the breeding season is higher than that of nonbreeding animals. The high tide values of the animals during both periods were subjected to Student's t test, and the difference between the two sets of values is statistically significant.

Oxygen uptake and tidal cycle

It is well known that periodic tidal inundations can shape behavioral and physiological responses into defined rhythms. Fluctuations in oxygen consumption over a period of time conform to tidal rhythm. Such results show three different patterns in the respiratory responses: T1, a neatly defined rhythm; T2, a tidal rhythm with additional peaks during low tides; or T3, absence of any defined rhythm correlated with phase of the tide. Data which are typical of the three
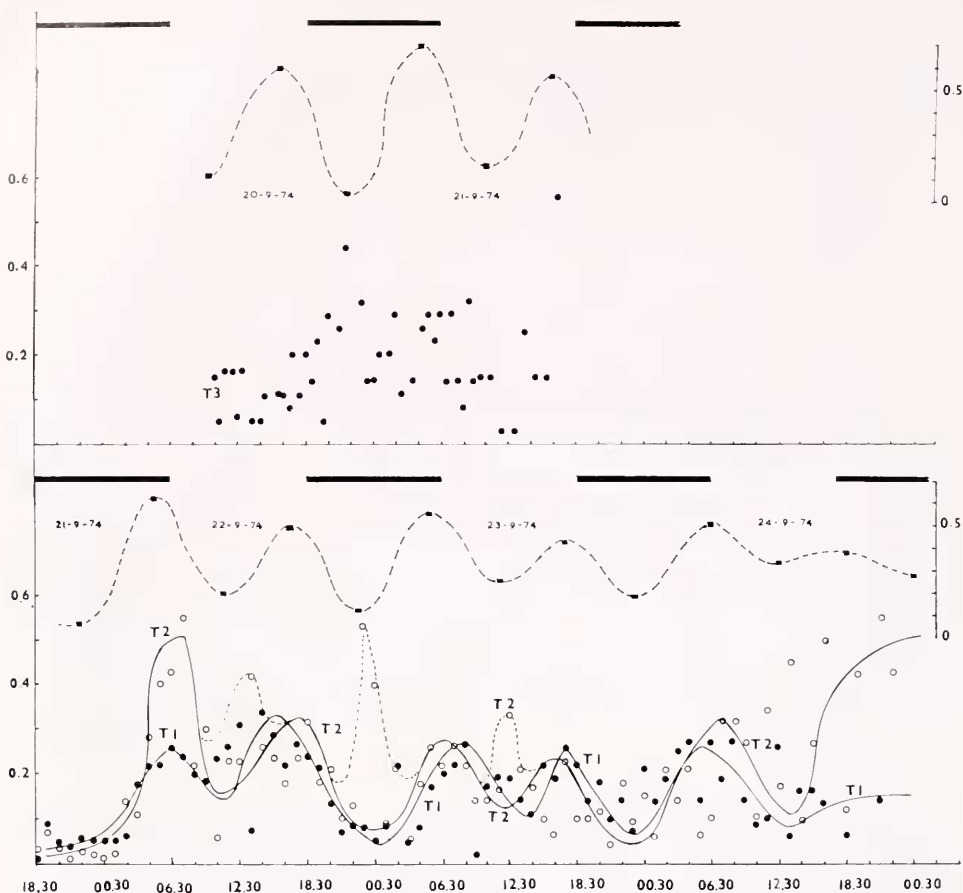
FIGURE 2.   Three types of respiratory responses described in the text (T1, T2, T3) of
P. flava to tidal cycle.   The abscissae represent the times of day in hours; and the ordinates,
rates of oxygen uptake in ml/hr.   Solid bars represent hours of darkness in the habitat; and
the ordinates in the upper right of each graph, the height of the tide in meters.

types of responses are illustrated in Figure 2, and for fifteen animals are given as
the average values of oxygen uptake during the successive low and high tide
periods in Table II.

Six out of fifteen specimens (T1) showed a rhythmicity in oxygen consump-
tion whose phases seem to synchronize with some points of the habitat tidal cycle.
The relationship between oxygen uptake and the tidal stages is direct, the rate
of oxygen consumption being high during high tide and low during low tide.

In a further five out of the fifteen specimens (T2), besides the occurrence of
a recurring rhythmicity, there was a peak value of oxygen uptake during low tide
period.   There remained four (T3) for which no rhythm could be detected.

Further, it is seen from Figure 2 that, when the height of the tide is lowest,
the corresponding low tide values are also lowest when compared with other low

TABLE II

Data on the oxygen uptake of Ptychodera flava during high tide and low tide: H represents high tide period; L, low tide period; Max, maximum amount of oxygen uptake, ml/hr; Min, minimum oxygen uptake, ml/hr; OAH, average oxygen uptake during high tide periods, ml/hr; OAL, average oxygen uptake during low tide periods, ml/hr; RAH, rate of oxygen uptake (ml/g/hr) during high tide periods; RAL, rate of oxygen uptake (ml/g/hr) during low tide periods. Dates of experiments for the animals were: 1 to 3 on January 23–24, 1974; 4 to 7, September 20–21, 1974; 8 to 11, September 21–24, 1974; 12 to 13, September 21–22, 1974; 14 to 15, September 22–24, 1974.

Oxygen uptake ml/hr

Animal number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Dry weight (mg)	190.6	227.0	130.2	251.2	197.0	60.8	46.4	117.0	121.4	130.0	30.4	219.0	94.2	50.0	51.0
Tidal phase															
H	0.10012	0.10841	0.06927	0.13765	0.28213	0.18570	0.07060	0.16613	0.09120	0.04478	0.04917	0.20142	0.19478	0.23610	0.11420
L	0.07353	0.03545	0.07660	0.13693	0.11264	0.16238	0.13001	0.37298	0.31395	0.32645	0.22468	0.25004	0.37145	0.20795	0.21506
L	0.12466	0.07846	0.08880	0.23580	0.15297	0.15844	0.21783	0.35076	0.44376	0.25812	0.21616	0.23653	0.33885	0.14976	0.22236
L	0.03670	0.08183	0.18480	0.24875	0.20333	0.22037	0.44717	0.34050	0.34566	0.23988	0.26985	0.22060	0.36576	0.11895	0.29745
H				0.13597	0.13375	0.17602	0.16650	0.27171	0.29620	0.24137	0.10715	0.15650	0.44717	0.10747	0.25967
L				0.28337	0.17380	0.18630	0.21490	0.33000	0.26330	0.20080	0.17110				
H								0.25543	0.14498	0.19993	0.13988				
L								0.24520	0.20420	0.12650	0.20488				
H								0.26518	0.21536	0.12708	0.14763				
L								0.24000	0.32736	0.19706	0.23241				
H								0.29476	0.22536	0.24473	0.15300				
L								0.26076	0.39500	0.40730	0.12516				
OAH	0.11239	0.09343	0.07903	0.22301	0.16325	0.18968	0.26402	0.29974	0.30824	0.24966	0.20468	0.23532	0.36860	0.16340	0.2562
RAH*	0.58910	0.41150	0.60690	6.88770	0.82860	3.11900	5.69000	2.56100	2.53900	1.92000	6.73200	1.07400	3.91200	3.26200	5.02300
OAL	0.05511	0.05864	0.13040	0.16980	0.18960	0.17338	0.14964	0.26716	0.21947	0.18600	0.13549	0.20010	0.32693	0.16444	0.19870
RAL*	0.28910	0.25830	1.00100	0.67590	0.96240	2.85100	3.22500	2.28300	1.80700	1.43000	4.45600	0.91360	3.47006	3.28800	3.89600
Min.	0.0205	0.01940	0.02050	0.03100	0.04880	0.07750	0.0132	0.04270	0.02440	0.00920	0.01320	0.05670	0.08710	0.03690	0.08970
Max.	0.2160	0.19220	0.13670	0.56680	0.44350	0.65010	0.432	0.57450	0.51850	0.55470	0.34780	0.43340	0.70720	0.22950	0.49120

* Data used in Figure 1.

<center>TABLE III</center>

Influence of salinity on the oxygen uptake of P. flava; the change from normal sea water (30.5‰)
to subnormal sea water (20.5‰) took place between 1600 and 1630 hours on January 24, 1974.
Data for the first three hours were also used in Figure 1.   Mean (H) and Rate (H) refer
to average oxygen uptake and rate of oxygen uptake during high tide periods.

Time	Oxygen uptake ml/hr				Summary		
						Oxygen uptake ml/hr	
	Animal 1 Dry wt 89.0 mg	Animal 2 Dry wt 140.0 mg	Animal 3 Dry wt 325.0 mg		Animal 1	Animal 2	Animal 3
13.00	—	0.0264	—		In normal sea water (30.5‰)		
13.30	0.1056	0.1608	0.1602				
14.00	0.0191	0.0191	0.0370	Mean (H)	0.0679	0.0811	0.1095
15.00	—	0.0864	—	Rate (H)	0.7629	0.5792	0.3363
15.30	0.0571	0.1075	0.0754	Min	0.0191	0.0191	0.0396
15.45	0.0756	0.0864	0.0935	Max	0.1056	0.1608	0.1814
16.00	0.0820	—	0.1814				
16.30	0.3067	0.1142	0.0972		In subnormal salinity (20.5‰)		
16.45	0.2102	0.2285	0.1085				
17.00	0.1401	0.1612	0.2277	Mean	0.1621	0.1358	0.1733
17.15	0.0969	0.0694	0.0874	Rate	0.1821	0.9700	0.5322
17.30	0.1180	0.2285	0.1404	Min	0.0643	0.0264	0.0217
17.45	0.1507	0.1080	0.1099	Max	0.3067	0.2285	0.3859
19.00	0.1392	0.0936	0.1837				
19.45	0.0643	0.0264	0.1814				
20.30	0.2958	0.1930	0.0630				
21.00	0.1366	0.0662	—				
22.00	0.1497	0.1242	0.0217				
22.45	0.1094	0.1613	0.2088				
00.00	0.1299	0.1005	0.3859				
1.00	0.2217	0.2261	0.2419				

tide values.   The rhythm seems to be persistent for about 65 hours, and then becomes irregular or not detectable.   Waning of the tidal rhythm was noticed in four animals studied for a period of 72 hours.

Salinity induced respiratory response

Data on the effect of salinity on oxygen uptake of three specimens are given in Table III.   The average readings for three animals weighing 89 mg, 140 mg, and 325.6 mg in habitat sea water (30.5‰) are 0.0679, 0.0811 and 0.109 ml/hr, respectively and in reduced salinity (20.5‰) they consume 0.162, 0.136 and 0.173 ml/hr.   The small specimen weighing 89.0 mg shows a difference of 0.094 ml/hr which is about 140% of the value obtained in habitat sea water; in the larger specimens, the increase being 66% (140 mg) and 58% (325.6 mg).   It seems probable that the smaller animals respond more sharply than the larger animals. Response to salinity stress tends to become stabilized in a period of about an hour.

Discussion

The synchronization of one or the other of the physiological processes in intertidal animals with the phase of recurring tide can result in a rhythmical temporal pattern, the tidal rhythm. A tidal rhythm of oxygen consumption seems to persist in *Ptychodera flava* under constant conditions in the laboratory. Many factors could be involved in the actual entrainment, including: locomotor activity, diurnal periodicity, tidal inundation of sea water including temperature changes, mechanical agitation, and chemical changes in the medium. In *Ptychodera flava*, since the size of the animal chamber was just big enough to house the animal, which is sluggish most of the time (Hyman, 1959), it is concluded that the periodic increase and decrease in oxygen consumption could not be attributed to the locomotor rhythms and that the peak time of oxygen consumption must be due to other factors. A circadian rhythm may persist in the absence of daily cycles of temperature or of light, with maximum consumption of oxygen during the day, and minimum during the night. Specimens of *Uca* and *Sesarma reticulatum* show both circadian and tidal rhythms (Barnwell, 1966; Palmer, 1967) in that besides coincidence of two peaks with the high water, there is a prominent nocturnal peak of locomotor activity. In *Emerita,* an exaggerated nightly activity was considered to be due to the superimposition of a diurnal rhythm on a tidal cycle (Chandrasekaran, 1965). A close observation of the results reported in the present study reveals a decline in oxygen consumption at night. Concomitant increase in oxygen consumption with high water in the early hours of the day is also evident. All of this suggests a tidal rather than a true diurnal rhythm.

This rhythm reflects the prevailing ecological conditions in the habitat. In the area enclosed by the Galaxea reef, it is significant that the lack of breaking waves and the reduction of water depth results at times in the exposure of patches of the substratum. Any corresponding variation in the activity of the animals (by deeper burrowing, for example) will involve a variation in the pattern of oxygen uptake. The pattern of oxygen consumption in five out of fifteen animals is slightly at variance with the previous pattern in that there is an increase in oxygen uptake during the low tide periods.

Laboratory observations show that *P. flava* does make nocturnal excursions on the substratum, mostly when the conditions of the habitat become unfavorable in terms of the availability of food (Azariah, unpublished). They react to environmental extremes by migrating up to the surface and lying passively. Probably, in the environment, they may be moved by the waves to other more favorable places under these conditions. Thus, the activity patterns appear more complex and influenced by more than one factor.

Four animals did not show any tidal rhythm. The observations were made on the four animals simultaneously, and hence their pattern is a reflection of the pattern of the tidal cycle prevailing during the time of those particular experiments. The absence of tidal rhythm may result from the necessity for "conditioning" the animal to the experimental set-up used. In data obtained towards the end of the experiments (after about 20 hours), the animals show a tendency to exhibit a rhythm.

Enright (1963) reported that the activity levels of the amphipod *Synchelidum* were related to the amplitude of the tides. The activity rhythm of the sand crab *Emerita asiatica* coincided with the amplitude of the tide (Chandrasekaran, 1965). A similar relationship in *P. flava*, together with the waning of the tidal rhythms with time, suggests that the rhythm is phased by the environmental variables.

It is interesting to note the relationship between salinity changes and metabolism of *P. flava*. It responds to subnormal salinity by an increase in the respiratory rate, agreeing with the reports of Schlieper (1929), and Potts and Parry (1964). Although the increased respiration may suggest a causal relation to requirements for osmotic work, Potts and Parry (1964) and Vernberg and Vernberg (1972) suggest that changes in salinity may alter the locomotor activity of an organism, and hence changes in metabolic rate may reflect behavioral changes rather than the effect of salinity on basic metabolic processes (see Duncan, 1966). In *P. flava* no attempt was made to study its behavioral response to salinity changes. The pronounced rise in the respiratory rate on sudden changes of salinity and its gradual decline followed by the maintenance of a higher level of oxygen consumption than that of the one prior to exposure to a new salinity regime, show its short term overshoot reaction and the longer continuing responses. There seems to be a conflict between the inherent tidal rhythm and salinity-induced changes in respiration; there is a tendency to keep to the rhythm of tide, but the influence of salinity changes is dominant.

Time at Krusadi Island was limited, data were processed at Madras, and further experiments were impossible. Further work, following the suggestions given by Kinne (1971), is now planned.

Summary

1. Some aspects of the ecology and respiratory physiology of *Ptychodera flava* have been studied.

2. Increase in body weight involves a decrease in the rate of oxygen consumption. At high tides, the rates of oxygen uptake of nonbreeding *P. flava* are 0.7629 (smallest) and 0.3363 (largest) in ml/g/hr. At high tides, mature and spawning female rates are 6.732 (smallest) and 0.8877 (largest) ml/g/hr. Rate of oxygen uptake is a function both of body size and of maturity stage in *P. flava*.

3. Respiratory responses of 15 specimens of *P. flava* fell into three classes: 1, showing a rhythm synchronized with tidal phases; 2, showing a tidal rhythm with additional peaks during low tides; or 3, with no detectable rhythm.

4. *P. flava* reacts to lowered salinity by showing a sudden rise in respiration. The sustained level of respiration is subsequently a little higher than in normal sea water.

5. The deep-seated tidal rhythm shows variations, and it is suggested that locomotor activity, spawning, temperature, and salinity may all be factors involved.

LITERATURE CITED

ASHWORTH, R. B., AND M. J. CORMIER, 1967. Isolation of 2,6-dibromophenol from the marine hemichordate, *Balanoglossus biminiensis. Science,* **155** : 1558–1559.

AZARIAH, J., 1969. Physiological and histochemical studies on some protochordates. *Ph.D. thesis, University of Madras,* India, 105 pp.

BARNWELL, F. H., 1966. Daily and tidal patterns of activity in individual fiddler crabs (Genus *Uca*) from the Woods Hole region. *Biol. Bull.,* **130** : 1–7.

BARRINGTON, E. J. W., 1940. Observations on feeding and digestion in *Glossobalanus minutus. Quart. J. Microscop. Sci.,* **82** : 227–260.

BARRINGTON, E. J. W., 1965. *The Biology of Hemichordata and Protochordata.* Oliver & Boyd, London, 176 pp.

CHANDRASEKARAN, M. K., 1965. Persistent tidal and diurnal rhythms of locomotory activity and oxygen consumption in *Emerita asiatica* (M. Edw.) *Z. Vergl. Physiol.* **50** : 137–150.

DE JORGE, F. B., AND J. A. PETERSEN, 1968a. Accumulation of "glycogens" in the genital region of *Balanoglossus gigas* Fr. Muller (Spengel, 1893) Enteropneusta during reproduction. *Comp. Biochem. Physiol.,* **26** : 737–740.

DE JORGE, F. B., AND J. A. PETERSEN, 1968b. Comparative biochemical studies on *Balanoglossus clavigerus* and *Willeyia loya* (Enteropneusta). *Comp. Biochem. Physiol.,* **27** : 551–557.

DE JORGE, F. B., J. A. PETERSEN, AND P. SAWAYA, 1967. Biochemical studies on the enteropneust *Balanoglossus gigas* (Fr. Muller, 1898). *Comp. Biochem. Physiol.,* **22** : 467–475.

DEVANESAN, D. W., AND P. I. CHACKO, 1942. *Balanoglossus* as food of fishes. *Curr. Sci.,* **11** : 242–243.

DUNCAN, A., 1966. The oxygen consumption of *Potomopyrgus jenkinsi* (Smith) (Prosolvanchiata) in different temperatures and salinities. *Verh. Int. Ver. Limnol.,* **16** : 1739–1751.

ENRIGHT, J. T., 1963. The tidal rhythm of activity of a sand-beach amphipod. *Z. Vergl. Physiol.,* **46** : 276–313.

FRY, F. E. J., AND J. S. HART, 1948. The relation of temperature to oxygen consumption in the gold fish. *Biol. Bull.,* **94** : 66–77.

HYMAN, L. H., 1959. *The Invertebrates,* Volume 5. McGraw-Hill Book Company, Inc., New York, 783 pp.

JAYARAMAN, N., 1954. Seasonal variations in salinity, dissolved oxygen and nutrient salts in the inshore waters of the Gulf of Mannar and Palk Bay near Mandapam (S. India). *Indian J. Fish.,* **1** : 345–364.

JOB, S. V., 1955. The oxygen consumption of *Salvelinus fontinalis. University of Toronto Biological Series,* **61** : 1–39.

KINNE, O., 1971. Salinity animals, invertebrates. Pages 821–995 in O. Kinne, Ed., *Marine Ecology,* Volume 1, Part 2. Wiley-Interscience, John Wiley & Sons Ltd., London.

KRISHNAN, G., AND P. GOVINDARAJULU, 1968. Observations on the biochemical composition of the enteropneust *Ptychodera flava. Life Sci.,* **7** : 629-632.

KURIYAN, G. K., 1949. On the occurrence of Enteropneusta in Shingle Island, Gulf of Mannar. *Curr. Sci.,* **18** : 258.

MACHA, N., 1969. A histochemical study of epidermal mucous secretion in Enteropneusta. *Acta Histochem.,* **34** : 305–309.

MUTHU, M. S., 1956. Studies on Plankton. *M.Sc. thesis, University of Madras,* Madras, India.

PALMER, J. D., 1967. Daily and tidal components in the persistent rhythmic activity of the crab, *Sesarma. Nature,* **215** : 64–66.

PETERSEN, J. A. AND L. LONGHI, 1971. Amino acid distribution in *Glossobalanus* (Ptychoderi-
cae, Enteropneusta Hemichordata). *Histochemistry,* **16**: 323–327.

POTTS, W. T. W. AND G. PARRY, 1964. *Osmotic and ionic regulation in animals.* Pergamon
Press, London, 423 pp.

RAMANUJAM, S. G. M., 1935. Enteropneusta from Krusadi Island. *Curr. Sci.,* **3**: 427.

RAO, K. P., 1954a. The early development of Enteropneusta *Ptychodera flava* Eschscholtz.
J. Zool. Soc. India, **6**: 145–152.

RAO, K. P., 1954b. Bionomics of *Ptychodera flava. J. Madras Univ.,* **24**B: 1–5.

RAO, K. P., 1955a. Enteropneusta from Madras and the Gulf of Mannar, with a note of the
probable course of distribution of *P. flava* Eschscholtz. *Proc. Indian Sci. Congr.,*
44: 301.

RAO, K. P., 1955b. Morphogenesis during regeneration in an Enteropneust. *J. Anim. Morphol.
Physiol.,* **1**: 1–7.

SCHLIEPER, C., 1929. Uber die Einwirkung neiderer Salzkonsentrationen auf marine Organis-
men. *Z. Verg. Physiol.,* **9**: 478–514.

SUNDARA RAO, P. J., AND S. RANGA RAO, 1949. A note on the occurrence of a giant Balano-
glossid at Krusadi Island. *J. Bombay Natur. Hist. Soc.,* **48**: 813.

UDAYAVARMA, T. P. AND C. V. GANGADHARA REDDY, 1959. Seasonal variations of the hydro-
logical factors of the Madras coastal waters. *Indian J. Fish.,* **6**: 298–305.

VERNBERG, W. B. AND F. J. VERNBERG, 1972. *Environmental physiology of marine animals.*
Springer-Verlag., Berlin, 346 pp.